CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

Spiromesifen

Chemical Code # 5858, **Tolerance #** 52945
SB 950 # NA

Original: December 17, 2003
Revised: February 1, 2005

I. DATA GAP STATUS

Chronic toxicity, rat:	No data gap, no adverse effect indicated.
Chronic toxicity, dog:	No data gap, no adverse effect indicated.
Oncogenicity, rat:	No data gap, no adverse effect indicated.
Oncogenicity, mouse:	No data gap, no adverse effect indicated.
Reproduction, rat:	No data gap, no adverse effect indicated.
Teratology, rat:	No data gap, no adverse effect indicated.
Teratology, rabbit:	No data gap, no adverse effect indicated.
Gene mutation:	No data gap, no adverse effect indicated.
Chromosome effects:	No data gap, no adverse effect indicated.
DNA damage:	No data gap, no adverse effect indicated.
Neurotoxicity:	Not required at this time.

Toxicology one-liners are attached.

All record numbers through 204964 were examined.
** indicates an acceptable study.
Bold face indicates a possible adverse effect.
indicates a study on file but not yet reviewed.
File name: T050201
Revised by Peter Leung, 2/1/05

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may contain additional effects.

COMBINED, RAT

Study not submitted.

CHRONIC TOXICITY, RAT

** 0098; 204869; Schladt, L., E. Hartmann, and A. Popp (2001) BSN 2060: Chronic toxicity study in Wistar rats (Dietary administration over 1 year). Bayer AG, Department of Toxicology, Wuppertal, Germany. Laboratory Project ID: Bayer AG Report No. PH 31617. Bayer AG Study No. T8068185. (Dec. 13, 2001). In a chronic toxicity study (MRID 45819621) Spiromesifen (purity, 97.0 to 97.6%, mixed batch No. 06957/0009) was administered to 25 Wistar rats/sex/dose in diet at dose levels of 0, 50, 125, 300, or 800 ppm (equivalent to 0, 2.6, 6.5, 15.9 or 42.4 mg/kg bw/day in males and 0, 3.0, 7.6, 19.3, or 51.7 mg/kg bw/day in females) for 1 year. In addition to standard elements of a chronic study, this study included functional observational battery (FOB) assessments for 10/sex/dose at weeks 25 and 52. Also, 5/sex/group were perfusion-fixed for neurohistopathology evaluation at termination, whereas the other 20 rats/sex/group received standard gross and histopathology examinations. Liver homogenates were evaluated for metabolic induction: 10/sex/dose were evaluated for aminopyrine-N-demethylase, p-nitroanisole-O-demethylase, and cytochrome P-450. UDP-glucuronyl transferase activity was evaluated in 5/sex/group. At each of the three intervals designated for hematology and clinical chemistry, hormones associated with thyroid function were assayed (T3, T4, TSH). At week 53, thyroxine binding capacity was also evaluated in the same rats. Treatments were well-tolerated, having no effects on survival, food consumption, nor FOB parameters. Body weight was marginally reduced in 800 ppm females only. Twenty rats/sex/group were evaluated for histopathology of thyroids and adrenals in all groups except for 125 ppm males (with N = 19). Thyroid follicular changes (hypertrophy and colloidal alteration) were the most remarkable changes. Follicular hypertrophy incidences in controls through progressively higher dose levels were 1, 1, 0, 8, and 9 for males, and 1, 2, 1, 0, and 11 in females. Colloidal alteration incidences in corresponding male groups were 10, 13, 11, 15, and 17 (the higher two group responses being significantly elevated when incidence and grade were considered). Colloidal alteration incidences in female controls through progressively higher dose levels were 1, 2, 1, 0, and 11. Most of these high dose female responses were one grade higher than the minimal grade typical of affected rats in other groups. Sporadic thyroid hormone level changes were consistent with thyroid histopathology findings. Elevations of T3 levels in 800 ppm males up to 162% of controls and in 300 ppm males up to 135% of controls were observed at week 27, but at week 14 only the high dose was significantly elevated, and there was no treatment effect evident at week 53. There was a general elevation of TSH over time (not statistically significant, but consistently elevated in both sexes at 800 ppm at all measurement intervals). T4 was unaffected in either sex. Adrenal gland microscopic changes were limited to high dose females, with cytoplasmic eosinophilia of the zona fasciculata incidences of 0, 0, 0, 0, and 7 in controls through 800 ppm females. This was observed grossly as a discoloration of the adrenals. At 800 ppm, both sexes had clinical chemistry changes consistent with altered liver function. These included significant decrements in bilirubin levels (68-87% of control levels in males, and 59-76% of control levels in females) and a general reduction in cholesterol (usually not significantly significant). Gross signs of swollen livers were seen in 800 ppm males, however there were no microscopic changes evident in livers. Activities of aminopyrine-N-demethylase and p-nitroanisole-O-demethylase were unaffected by treatment. Cytochrome P-450 levels were slightly elevated in 800 ppm females (significant, $p < 0.01$), but there was no change in corresponding males. UDP-glucuronyl transferase activity was unaffected in either sex. A few additional findings were mildly suggestive of high dose effects. Some of these were plausibly treatment-related, but were less convincing than the above findings based on statistical considerations and/or lack of consistency with other studies such as the primary rat oncogenicity study (Bayer AG Study No. T3067505, MRID 45819624): posterior capsular lens opacities in 800 ppm males, slight uterine endometrial inflammation at 800 ppm, and slightly increased severity of hypertrophy of uterine epithelium at 800 ppm. **The LOAEL in males is 300 ppm (15.9 mg/kg/day), based on the above thyroid follicular changes. The**

LOAEL in females is 800 ppm (51.7 mg/kg/day), based on thyroid follicular changes, cytoplasmic eosinophilia in the zona fasciculata of the adrenal cortex, and altered liver function (increased cytochrome P-450 concentration, decreased circulating levels of bilirubin, cholesterol, and total protein). Respective NOAEL's are 125 ppm (6.5 mg/kg/day) in males and 300 ppm (19.3 mg/kg/day) in females. This chronic study in the rat is **acceptable**, with no major deficiencies from guidelines, and satisfies the **guideline** requirement for a chronic oral study [OPPTS 870.4100, OECD 452] in rats. (Aldous, 6/19/03)

CHRONIC TOXICITY, DOG

** 0097; 204868; Ruf, J. and Sander, E., (2002) "Chronic toxicity study in beagle dogs (53 week feeding study)". Bayer AG, Department of Toxicology, Wuppertal, Germany. Laboratory Project ID: Bayer Corporation Agricultural Division Report No.G200102, Bayer AG Report No. PH 31665. Bayer AG Study No. T0069357. (Jan. 10, 2002). In a chronic toxicity study (MRID 45819620), Spiromesifen, 97.3 to 97.4% purity, Mixed batch No. 06957/0009, was administered to 4 purebred beagles/sex/dose in the diet at dose levels of 0, 50, 400, or 4000 ppm [equivalent to 0, 1.4, 11.5, or 109 mg/kg bw/day (M) or 0, 1.4, 10.8, or 117 mg/kg bw/day (F)] for 53 weeks. **The LOAEL is 11.5 mg/kg/day (M) or 10.8 mg/kg/day (F), based on induction of liver metabolic capacity: increased aminopyrine-N-demethylase and p-nitroanisole-O-demethylase activities, and increased cytochrome P-450 levels in liver tissue. Associated NOAEL is 1.4 mg/kg/day (both sexes).** These findings were much more pronounced at the high dose levels (109 and 117 mg/kg/day, respectively, in M and F), in addition to increased liver weights (37% increased in males, 18% in females), liver cytoplasmic change (4/4 males, 3/4 females, vs. none in other groups), consistent 2-fold to 4-fold increases in plasma alkaline phosphatase, and modest decreases in thyroxin (T4) (to about 50% of control levels). A transient clinical observations finding plausibly associated with treatment was that 3 of 4 of the 117 mg/kg/day females vomited at least once during the first week of treatment (emesis was otherwise comparatively uncommon in this study), yielding an "acute effects" NOAEL of 10.8 mg/kg/day in females for clinical observations. This chronic study in the dog is **acceptable, (guideline)** and satisfies the guideline requirement for a chronic oral study [OPPTS 870.4100, OECD 452] in the dog. (Aldous, 9/23/03)

ONCOGENICITY, RAT

** 0101; 204872; Schladt, L., (2001) BSN 2060: Carcinogenicity study in Wistar rats (Dietary administration over 2 years). Bayer AG, Department of Toxicology, Wuppertal, Germany. Laboratory Project ID: Bayer AG Report No. PH 31628. Bayer AG Study No. T3067505. (Dec. 10, 2001). In a carcinogenicity study (MRID 45819624) Spiromesifen, 97.0 to 97.6 % a.i., Mixed batch No. 06957/0009, was administered to 50 Wistar (Hsd Cpb:WU) rats/sex/group in diet at dose levels of 0, 50, 125, 300, or 800 ppm (equivalent to 0, 2.5, 6.1, 15, and 40 mg/kg bw/day in males or 0, 3.3, 8.2, 20, or 54 mg/kg bw/day in females) for 24 months. **The LOAEL is 15 mg/kg/day (M) based on increased posterior capsular lens opacity incidences, or 20 mg/kg/day (F), based on thyroid alteration [clumped colloid: significant ($p < 0.05$) by Mann-Whitney U test considering incidence and degree of change] and slight reduction of cholesterol levels in females (19% to 29% lower than controls between 52 weeks and 104 weeks, but statistically significant only at one of three sampling intervals).** The basis of the LOAEL in males was an increase in the most commonly reported lenticular lesion (posterior capsular opacity), with percentage incidences in males of 28, 45, 38, 60, and 52%. Incidence at 15 mg/kg/day was near to the upper range of historical control values, and was considered to be treatment-related by the study ophthalmologist. DPR reviewer accepts this LOAEL with the understanding that this is an equivocal finding, which was not substantiated by ocular histopathology. In contrast, thyroid follicular changes observed in females in this study are consistent with firmly established treatment effects in both sexes in the 1-year chronic dietary rat study [EPA MRID 45819621] at 300 and 800 ppm, suggesting that thyroid changes in females in the present study provide the best basis for NOAEL placement. Prominent findings at 800 ppm included modest and largely transient body weight decrements (a maximum of 6% in males and 7% in females during the first 9 weeks of the study, diminishing to 3-4% by week 25), and an increase in uterine dilatation (32/50 at 800 ppm, compared to 18/49 in controls). At the doses

tested, there was **not** a treatment related increase in tumor incidence when compared to controls. The presence of renal liposarcomas (uncommon tumors) in two 800 ppm males, versus none in any other groups, was nevertheless discussed in this review. Dosing was considered adequate based on body weight changes, and upon evidences of the above dose-responses. This carcinogenicity study in the rat is **Acceptable/Guideline**, and satisfies the requirement for a carcinogenicity study [OPPTS 870.4200; OECD 451] in rats. (Aldous, 8/11/03)

NOTE: Designation of "NOAEL" values in this study is provided for consistency with U. S. EPA conventions. For purposes of Cal-EPA classification, these results are **not** flagged as "possible adverse effects" to identify priority status for future risk assessment.

ONCOGENICITY, MOUSE

** 0102; 204873; Schladt, L., (2001) "BSN 2060: Oncogenicity study in CD-1 mice (Dietary administration over 18 months)." Bayer AG, Department of Toxicology, Wuppertal, Germany. Laboratory Project ID: Bayer AG Report No. PH 31622. Bayer AG Study No. T6068318. (Dec. 13, 2001). In a carcinogenicity study, MRID 45819625, Spiromesifen, 97.0 to 97.6 % a.i., Mixed batch No. 06957/0009, was administered to 50 CD-1 mice/sex/dose in diet at dose levels of 0, 20, 140, 1000, or 2000 ppm [equivalent to 0, 3.3, 22, 157, or 335 mg/kg bw/day (M) or 3.8, 30, 200, or 401 mg/kg bw/day (F)] for 18 months. **The LOAEL is 140 ppm, based on definitive and dose-related elevated incidences of cytoplasmic eosinophilia of the adrenal cortical zona fasciculata in both sexes. There were also sharp reductions in aging-related changes in adrenal cortex, such as diffuse fatty change and ceroid deposits in both sexes at 140 ppm and above [22 mg/kg/day (M) or 30 mg/kg/day (F)]. The corresponding NOAEL's are 3.3 mg/kg/day (M) and 3.8 mg/kg/day (F). Equivocal treatment-related alterations in amyloidosis in several tissues at 140 ppm and above were discussed in the DPR data review, however such findings are poorly suited for setting the LOAEL.** At the doses tested, there was not a treatment related increase in tumor incidence when compared to controls. Dosing was considered adequate based on dose-responses noted above. This carcinogenicity study in the mice is **acceptable** and satisfies **guideline** requirement for a carcinogenicity study [OPPTS 870.4200; OECD 451] in mice. (Aldous, 8/13/03)

REPRODUCTION, RAT

** 0096; 204867; Eiben, R., U. Bach, and M. Rinke, "BSN 2060: Two-generation study in Wistar rats," Bayer AG, Department of Toxicology, Wuppertal, Germany. Laboratory Project ID: Bayer Corporation Agricultural Division Report No. 200100, Bayer AG Report No. PH 31775. (Feb. 13, 2002). In a 2-generation reproduction study (MRID 45819619), Spiromesifen, 97.3 % a.i., Mixed batch No. 06957/0009 was administered to 25 Wistar rats/sex/dose in diet at dose levels of 0, 30, 120, or 500 ppm (equivalent to mean pre-mating intakes of 0, 2.2, 8.8, or 37 mg/kg bw/day in P males or 0, 3.8, 14.2, or 64 mg/kg bw/day in P females, or to pre-mating intakes of 0, 3.3, 13.2, or 76 mg/kg/day in F_1 males and 0, 4.6, 18.0, or 91 mg/kg/day in F_1 females). There was one mating period per generation. **The parental systemic LOAEL is 120 ppm (13.2 mg/kg bw/day in males, and 18.0 mg/kg bw/day in females), based on body weight decrements (of 6% and 17% at sacrifice of 120 ppm and 500 ppm F_1 adult males: 5% and 17% at sacrifice of corresponding F_1 females). The parental systemic NOAEL is 30 ppm (3.3 mg/kg bw/day in males, and 4.6 mg/kg bw/day in females). Some histopathology effects in 500 ppm parental rats, in most cases not statistically significant but consistent with observations of other rat long-term studies, included liver hypertrophy and periportal basophilia in F_1 females, thyroid follicular hypertrophy and colloidal alteration (primarily in F_1 rats), and elevated atrophy of the zona glomerulosa of the adrenal cortex (statistically significant in P females). There was a 15% increase in the numbers of primordial follicles in ovaries of 500 ppm F_1 parental females, which was possibly associated with general delayed growth, development, and maturation in these rats. The offspring LOAEL is 120 ppm (18.0 mg/kg bw/day), based on pup body weight decrements during lactation (decrements at lactation day 21 for 120 ppm and 500 ppm pups, respectively, were: 13.6% and 36.7% for F_1 males, 14.0% and 37.7% for F_1 females, 11.1% and 32.0% for F_2 males, and 8.4% and 31.9% for F_2**

females). Sexual maturation was delayed in F_1 500 ppm pups: 6 days for preputial separation and 3 days for vaginal opening. Body weights of 500 ppm males and females lagged behind contemporary controls by about 8 days, so that no primary reproduction effects are inferred by the maturation delays. The offspring NOAEL is 30 ppm (4.6 mg/kg bw/day). The reproductive NOAEL is 500 ppm (the highest dose tested: 76 mg/kg bw/day in males, and 91 mg/kg bw/day in females). There was no evidence of reproductive toxicity in this study. This study is acceptable, (guideline) and satisfies the guideline requirement for a 2-generation reproductive study (OPPTS 870.3800; OECD 416) in rats. (Aldous, 12/8/03)

0090; 204861; Eiben, R. and U. Bach, [2001]. "BSN 2060: Two-generation study in Wistar rats," (Aug. 24, 2001). Bayer Corporation Agricultural Division Report No. G110918, Bayer AG Study No. T 1068962. In a 2-generation reproduction study (MRID 45854511), Spiromesifen, 97.6 % a.i., Mixed batch No. 06957/0009 was administered to 25 Wistar rats/sex/dose in diet at dose levels of 0, 30, 120, or 500 ppm (equivalent to mean pre-mating intakes of 0, 2.6, 10.2, or 46 mg/kg bw/day in P males or 0, 3.3, 14.7, or 56 mg/kg bw/day in P females, or to pre-mating intakes of 0, 3.1, 13.6, or 58 mg/kg/day in F_1 males and 0, 4.7, 20.5, or 86 mg/kg/day in F_1 females). There was one planned mating period per generation, however a second mating of F1 parents was undertaken in this study due to poor lactation indices in the F2a mating period. The second mating (F2b) likewise produced low lactation indices. This study was taken to completion and fully reported, however a replacement study was subsequently undertaken [Eiben, R., U. Bach, and M. Rinke, (2002), Bayer Corporation Agricultural Division Report No. 200100, MRID 45819619, DPR Record No. 204867]. The replacement study did not have the problem with low lactation indices, and meets guideline requirements, and should be considered as the primary reproduction study for regulatory purposes. Data from the present study (MRID 45854511) have been examined by this reviewer primarily to compare and contrast with the primary study. The unacceptably low lactation indices in the present study suggested that there might be a critical weakness in the test animal stock. For this reason, a different source of parental rats was used in the replacement study: rats from the present study were from Harlan Winkelmann Borchen, Germany, whereas the source for the replacement reproduction study was Charles River Deutschland, (Sulzfeld). A minor difference between these two studies was that all histopathology in the present study evidently by Dr. U. Bach. Nearly all histopathology in the replacement study was performed by Dr. M. Rinke (only the quantitative ovarian follicle studies in that study were by Dr. Bach). Thus minor differences in reported responses of the two studies could be due to different test animal populations, or in the case of histopathology outcomes, to the subjective judgements of different pathologists as to what constitutes notable findings. Because the present study was replaced by another which was intended to be the definitive study, and because the two studies were comparable in essential design and conduct except as noted above, only an executive summary of the present study is being provided by DPR at this time. NOAEL's and LOAEL's below represent the F1 parental pre-mating test article exposures, which are slightly higher than P generation premating exposures on a mg/kg/day basis. This is consistent with the minimal toxicity evident in P males (no body weight effects, and no histopathology findings except for statistically non-significant alterations in incidences of thyroid findings suggestive of treatment responses). The P generation rats in this study only showed significant body weight decrements among lactating females, at which time actual food intake was probably substantially underestimated by pre-mating consumption. Further, the histopathology evident in P females may have reflected the heightened spiromesifen exposure during lactation, which immediately preceded sacrifices of these rats. **The parental systemic LOAEL is 500 ppm (58 mg/kg bw/day in F1 males, and 86 mg/kg bw/day in F1 females), based on body weight decrements (as high as 9% in lactating P females, and 8% and 9% at the end of the pre-mating period at week 12 in 500 ppm F_1 adult males and females, respectively). Additional findings in parent rats included slight increases in incidences of histopathology in thyroid gland (general increases, usually not statistically significant, of follicular cell hyperplasia and colloidal alteration in both sexes of both generations), adrenal cortex [increased incidence of "decreased vacuolation" in the zona glomerulosa (highly significant, but limited to P females only)], and possibly liver (decreased incidence of "decreased periportal fat content", only in P females). Although there was no consistent relative organ weight alteration across generations, a 9% decreased relative kidney weight in P males**

was consistent with findings of the replacement study, and might represent a minor treatment effect. The parental systemic NOAEL is 120 ppm (13.6 mg/kg bw/day in males, and 20.5 mg/kg bw/day in females). The offspring LOAEL is 500 ppm (86 mg/kg bw/day), based on pup body weight decrements during lactation (decrements compared to concurrent controls at lactation day 21 for 500 ppm pups were: 21% for F_1 males, 18% for F_1 females, 13% for F_2 males, and 24% for F_2 females). Sexual maturation criteria (preputial separation or vaginal opening) were unaffected in this study, in contrast to the results of the replacement study. This may reflect the comparatively small body weight decrements in pups in this study (about 2 to 3 day body weight gain delays at 500 ppm in either generation in this study, compared to about 8 days in the replacement study). The offspring NOAEL is 120 ppm (20.5 mg/kg bw/day). The reproductive NOAEL is 500 ppm (the highest dose tested: 58 mg/kg bw/day in males, and 86 mg/kg bw/day in females). There was no evidence of reproductive toxicity in this study. This study is **supplemental** to the above-cited replacement 2-generation reproductive study (MRID 45819619, DPR Record No. 204867), with respect to study type: (OPPTS 870.3800); OECD 416 in rats. Since essential NOEL's in this study were at or above those of the replacement study, there is no apparent reason to utilize the present study to represent key endpoints relating to reproductive effects. (C. Aldous, 11/7/03)

TERATOLOGY, RAT

** 0088; 204859; A.M. Klaus. (2001). BSN 2060: Developmental Toxicity Study in Rats After Oral Administration. Bayer AG, Department of Toxicology, D-42096 Wuppertal, Germany. Report No. 110844. September 7, 2001. In a developmental toxicity study (MRID 45819612), BSN 2060 Technical (purity: 97.6%, batch no. 06957/0009) was administered to 25 Wistar rats/dose by gavage at dose levels of 0, 10, 70, or 500 mg/kg bw/day from days 6 through 19 of gestation. One female in the 500 mg/kg treatment group was found dead on day 15. Three other females in this group demonstrated saltatory spasms intermittently up to 3.5 hours post-dose. The dams in the 70 and 500 mg/kg treatment groups demonstrated treatment-related effects of lower mean body weight gain and reduced food consumption ($p<0.05$ or 0.01). **The maternal LOAEL is 70 mg/kg bw/day, based on the lower body weight gain and reduced food consumption noted for this treatment group. The maternal NOAEL is 10 mg/kg bw/day**. In the fetal evaluation, there was no apparent treatment-related effect on fetal deaths or resorptions. Although the mean fetal body weight for the 500 mg/kg treatment group was slightly less than that of the control, it was not of clinical significance. There were no treatment-related effects noted for external or visceral abnormalities. In the skeletal examination, the incidences of an enlarged fontanelle, unossified hyoid bone and incompletely ossified parietal and interparietal bones were more common for the control fetuses than for the treated ones in a dose-related manner based on both fetal number and litter ($p<0.05$ or 0.01). A greater incidence of incomplete phalangeal ossification was likewise noted for the controls than for the treated fetuses based on fetal number and litter ($p<0.05$ or 0.01). Only for the cervical vertebral bodies was there an increased dose-related incidence of incomplete ossification and this was based only on fetal number ($p<0.05$ or 0.01) and not on litter. The increased incidence of greater ossification does not indicate a toxicological-relevant endpoint particularly in the absence of any osteopathic effects. **The developmental NOAEL is 500 mg/kg bw/day, based on the lack of treatment-related toxicity at the highest dose tested.** The developmental toxicity study in the rat is classified as **acceptable, guideline** and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in the rat. (Moore, 11/19/03)

TERATOLOGY, RABBIT

** 0077; 204820; B. Holzum. BSN 2060: Developmental Toxicity Study in Rabbits after Oral Administration (Amendment Attached). Report No. 110810. August 1, 2000, Amended, February 12, 2001. Bayer AG, Department of Toxicology, D-42096 Wuppertal, Germany.

In a developmental toxicity study (MRID 45819604) BSN 2060 Technical (purity: 97.4%, batch no. 06957/0009) was administered orally to 22 female CHBB:HM rabbits/dose by gavage at dose levels of 0, 5, 35 or 250 mg/kg bw/day from days 6 through 28 of gestation. Four females in the 250 mg/kg treatment group aborted between days 20 and 25 of gestation. These animals exhibited cold ears, severely decreased food consumption accompanied by body weight loss. Gross examination of these females revealed two of them having tightly filled stomachs and one with a light discolored small intestine. Microscopic examination of the latter lesion indicated a marked vacuolation at the villous tips. Cold ears were noted for most of the other females in this treatment group. Two other females suffered total resorption of fetuses. Transient reduced food consumption and body weight losses were noted for the 35 mg/kg treatment group as well. Otherwise, no deaths occurred during the study and no treatment-related lesions were noted in the gross necropsy except for those which had aborted. **The maternal LOAEL is 35 mg/kg bw/day, based** on transient body weight loss and reduced food consumption. **The maternal NOAEL is 5 mg/kg bw/day.** There was an increased number of late resorptions in the 250 mg/kg group because two females in this group suffered total resorptions. This effect is likely due to maternal toxicity evident in this treatment group. Otherwise, there were no fetal deaths and a comparable number of resorptions per litter for all of the study groups. There was no treatment-related incidence of malformations. Among the skeletal deviations observed specific bones either demonstrated greater or lesser ossification in a dose-related manner. However, the percentage of affected fetuses at any dose level was within the range or was comparable to the lower end of the range for the historical controls. The litter incidence for any of these effects in any treatment group also was not statistically significant from that of the control group. Therefore, these results were not considered to be treatment-related. **The developmental NOAEL is 250 mg/kg bw/day.** The developmental toxicity study in the rabbit is classified **acceptable guideline** and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in the rabbit. (Moore, 11/13/03)

GENE MUTATION

0059; 204802; B. Herbold. (1997). BSN 2060: *Salmonella*/Microsome Test Plate Incorporation and Preincubation Method. Bayer AG, Department of Toxicology, D-42096 Wuppertal, Germany. Report No. 109633. (October 10, 1997). In a reverse gene mutation assay in bacteria (MRID 45819516), strains TA 98, TA 100, TA 102, TA 1535, and TA 1537 of *S. typhimurium* were exposed to Technical BSN 2060 (99.4% a.i., batch no. NLL 5982-8A, dissolved in DMSO) at concentrations of 16 to 5000 µg/plate in the presence and absence of mammalian metabolic activation for 48 hours at 37^{o} C. In the 1st trial, the plate incorporation method was used. In the 2nd trial, the samples were preincubated for 20 minutes at 37^{o} C, followed by plate incorporation and incubation for 48 hours at 37^{o} C. Each treatment level was assayed in triplicate. An S9 fraction from Aroclor 1254-induced rat liver was used to metabolize the test material. As a means to assess cytotoxicity, a separate culture was performed in duplicate in which each treatment level was incubated in the presence of histidine under the same conditions as the mutagenicity assay and the bacterial titer was measured. The positive controls were functional under conditions of both activation and non-activation. **There was no evidence of a concentration-related positive response of induced mutant colonies over background. This study is classified as **acceptable guideline** and satisfies the guideline requirement for the requirement for Test Guideline OPPTS 870.5100; OECD 471 for *in vitro* mutagenicity (bacterial reverse gene mutation) data. (Moore, 10/3/03)

** 0063; 204806; B. Herbold. (1999). BSN 2060: V79-HPRT Test *In Vitro* for the Detection of Induced Forward Mutations. Bayer AG, Department of Toxicology, D42096 Wuppertal, Germany. Report No. 109637. April 26, 1999. In a mammalian cell gene mutation assay, HPRT locus, (MRID 45819520), hamster lung V79 cells cultured *in vitro* were exposed to Technical BSN 2060 (batch no. 06957/0009, a.i.: 97.4%, dissolved in ethanol) at concentrations of 1, 2.5, 5, 10, 15, 20, and 25 µg/mL in the absence and 10, 20, 35, 50, 65, 80, and 95 µg/mL in the presence of mammalian metabolic activation with Aroclor 1259-induced rat liver S9 fraction for 5 hours at 37^{o} C. Two cultures per treatment level and two trials were performed. For the cytotoxicity evaluation, Technical BSN 2060 was tested at concentrations up to 125 µg/mL under conditions

of activation and non-activation. At 100 µg/mL and above, precipitation of the test material was noted in the culture medium. At 25 µg/mL and above in the absence of activation, the relative cloning efficiency ranged between 2.8 and 15.1% in a non-dose related manner. For the activated assay, the relative cloning efficiency of the 50, 100 and 125 µg/mL treatment levels were 39.5, 2.7 and 0.6%, respectively. The positive controls induced the appropriate response. **There was no concentration-related positive response of induced mutant colonies over background under conditions of activation or non-activation.** This study is classified as **acceptable (guideline)** and satisfies the guideline requirement for Test Guideline OPPTS 870.5300, OECD 476 for *in vitro* mutagenicity (mammalian forward gene mutation) data. (Moore, 10/15/03)

CHROMOSOME EFFECTS

** 0060; 204803; B. Herbold. (1997) *In Vitro* Mammalian Chromosome Aberration Test with Chinese Hamster V79 Cells. Bayer AG, Department of Toxicology, D-42096 Wuppertal, Germany. Report No. 109634. December 1, 1997. In a mammalian cell cytogenetics assay *[Chromosome aberration]*(MRID 45819517), Chinese Hamster V79 cell cultures were exposed to Technical BSN 2060 (98.8 to 99.4% a.i., batch no. NLL 5982-8A, dissolved in DMSO) at concentrations of 0 (negative control), 0 (vehicle control), 10, 20, 40, 60 and 80 µg/mL with metabolic activation and 0 (negative control), 0 (vehicle control), 1, 5, 10, 20, and 40 µg/mL without metabolic activation for 4 hours, followed by 14 hours of additional incubation. In addition, the cells were exposed to treatment levels of 0 (vehicle control), 40, 60 and 80 µg/mL with metabolic activation and 0 (vehicle control), 10, 20, and 40 µg/mL without metabolic activation for 4 hours, followed by 26 hours of incubation. Two hours prior to the cell harvest, 0.2 ml of Colcemid solution (40 ug/ml) was added to each culture. Cultures were performed in duplicate. Mitotic and survival indices were determined for each of the treatment levels. Chromosomal aberrations were evaluated according to the classification process of Rieger and Michaelis (1967). Cytotoxicity was noted at 10 ug/ml and above for the non-activated assay and at 20 ug/ml and above for the activated assay. Positive controls induced the appropriate response. **There was no evidence of a concentration related positive response of chromosomal aberration induced over background.** This study is classified as **acceptable (guideline)** and satisfies the guideline requirement for Test Guideline *In vitro* mammalian cytogenetics OPPTS 870.5375; OECD 473 for *in vitro* cytogenetic mutagenicity data. (Moore, 10/8/03)

DNA DAMAGE

** 0062; 204805; B. Herblod. (1999). BSN 2060: Micronucleus Test on the Male Mouse. Bayer AG, Department of Toxicology, D-42096 Wuppertal, Germany. Report No. 109636. April 16, 1999. In a mouse bone marrow micronucleus assay (MRID 45819519), 5 male mice/dose were treated by intraperitoneal injection with Technical BSN 2060 (batch no. 06957/0009, a.i.: 97.4%) at doses of 0, 100, 200 or 400 mg/kg bw twice over a 24 hour period. Bone marrow cells were harvested at 24 hours post-treatment. The vehicle was 0.5% aqueous Cremophor. The following signs of toxicity observed during the study: apathy, roughened fur, loss of weight, sternal recumbency, spasm, extension spasm, difficulty breathing, and slitted eyes. No animals died. The test material was tested at an adequate dose based on the treatment-related signs at all of the dose levels. The positive control induced the appropriate response. **There was no significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow 24 hours after treatment.** This study is classified as **acceptable (guideline)** and satisfies the guideline requirement for Test Guideline OPPTS 870.5395; OECD 474 for *in vivo* cytogenetic mutagenicity data. (Moore, 10/9/03)

NEUROTOXICITY

52945-0082; 204825; R.G. Gilmore and L.P. Sheets. (2001). An Acute Oral Neurotoxicity Screening Study with Technical Grade BSN 2060 in Wistar Rats. Bayer Corporation, Agricultural Division, Toxicology, Stilwell, KS. Report No. 110815. (December 10, 2001).

In an acute neurotoxicity study (MRID 45819606*)*, groups of 5 fasted 9-week old Wistar rats per sex per dose were given a single oral dose of technical grade BSN 2060 (mixed batch no. 06957/0009, purity = 97.1%) in 0.5% methyl cellulose/0.4% Tween 80 in deionized water at doses of 0 (vehicle only), 200, 700, and 2000 mg/kg bw and observed for14 days. Neurobehavioral assessment (functional observational battery and motor activity testing) was performed in 12 animals/sex/group one week prior to treatment, approximately 4 hours after treatment, and 7 and 14 days after treatment. At study termination, 6 animals/sex/group were euthanized and perfused *in situ* for neuropathological examination. Of the perfused animals, males and females in the control and high-dose groups were subjected to histopathological evaluation of brain and peripheral nervous system tissues. Treated-related urine staining was observed in 1 female at 700 mg/kg and in 3 females at 2000 mg/kg on Day 0. A treated-related decrease in mean total motor and locomotor activity was observed in females at 2000 mg/kg on Day 0 (without statistical significance), returning to control levels on Days 7 and 14. There were no treatment-related effects on mortality, body weight, brain weight, gross and histological pathology, or neuropathology. FOB testing revealed no treatment-related effects. NOEL (M) = NOAEL (M) = 2000 mg/kg (based on no effects at the highest dose tested). NOEL (F) = NOAEL (F) = 200 mg/kg and LOAEL (F) = 700 mg/kg (based on clinical signs- urine staining). This neurotoxicity study is classified as an **Unacceptable/Guideline study** and does not satisfy the guideline requirement for an acute neurotoxicity study in rats (870.6200; OECD 424). The study may possibly be upgraded to acceptable with the submission of positive control data generated within a few years of the time interval of the study in review. (Corlett, 8/28/03)

52945-0083; 204826; R.G. Gilmore, L.P. Sheets and S.G. Lake. (2002). A Subchronic Neurotoxicity Screening Study with Technical Grade BSN 2060 in Wistar Rats. Bayer Corporation, Agricultural Division, Toxicology, Stilwell, KS. Report No. 110820. (January 15, 2002). In a subchronic neurotoxicity study (MRID 45819607), Technical Grade BSN 2060 (% a.i. 97.1, batch/lot #06957/0009) was administered to 12 Wistar rats/sex/group at dose levels of 0, 100, 500, or 2000 ppm (equivalent to (M) 0, 6.4, 31.8, 122.7 mg/kg bw/day, (F) 0, 7.9, 38.3, 149.3 mg/kg bw/day) for 13 weeks. Neurobehavioral assessment (functional observational battery and motor activity testing) was performed in 12 animals/sex/group prior to treatment initiation and after 4, 8 and 13 weeks of treatment. At study termination, 6 animals/sex/group were euthanized and perfused *in situ* for neuropathological examination. Of the perfused animals, 6/sex/group in the control and high dose groups were subjected to histopathological evaluation of brain and peripheral nervous system tissues. No deaths resulted from the treatment. No treatment-related effects were noted for the 100 and 500 ppm treatment groups. The mean body weights of the 2000 ppm animals were lower than that of the controls over the course of the study (males: 93% of control, females: 91% of control). Likewise, food consumption was lower for both sexes in this treatment group (males: as low as 93% of control, females: as low as 85% of control). In the FOB, one female in the high dose group after 13 weeks of treatment exhibited a more energetic response to auditory and tail pinch stimuli and a rigid body tone on handling. The investigators identified this response as treatment-related based on a pattern of aggressive behavior observed in rats treated at 3000 ppm in another subchronic feeding study (report no. 109632). The minimal aspect of these responses and the one time observation in one animal over a 13 week treatment period makes this conclusion questionable. An apparent treatment-related effect in the foot splay measurement was noted for the animals in the 2000 ppm treatment group in which they exhibited a reduced mean splay (males: week 8, females weeks 4, 8 and 13) (NS). The particular neurological significance of this finding is unknown as the test material used as a positive control to evaluate this parameter, acrylamide, results in a significant broadening of the splay distance. No other parameter (e.g. grip strength, righting reflex, neuropathology) demonstrated any effect resulting from the treatment. There were no other apparent treatment-related effects in the FOB, motor activity measurements and neuropathology. **Based on the effects seen in this study, the LOAEL was 2000 ppm ((M) 122.7 mg/kg/day, (F) 149.3 mg/kg/day) (based on the lower mean body weights and food consumption of the 2000 ppm treatment group), with a NOAEL of 500 ppm ((M) 31.8 mg/kg/day, (F) 38.3 mg/kg/day).** The study is classified as **unacceptable and does not satisfy the guideline requirement** for a subchronic neurotoxicity study in rats (870.6200b). The frequency of diet preparation, the storage conditions and the test data for the homogeneity and stability of the diet preparations were not presented in the report. In

addition, only the mean analytical concentrations of the test material in the dietary preparations determined over the course of the study were reported. These deficiencies can possibly be corrected with the submission of documentation which adequately details these points of concern. (Moore, 9/24/03)

SUBCHRONIC STUDIES

(4-week rat feeding study)

52945-0057; 204800; F. Krotlinger and E. Sander. (1998). BSN 2060 Study for Subacute Oral Toxicity in Rats (Feeding Study for 4 Weeks). Bayer AG, Department of Toxicology, Wuppertal, Germany. Report No. 109631. (September 10, 1998). In a 4-week oral toxicity study (MRID 45854505), BSN 2060 (Batch No. NLL 5982-11, purity = 100%) was administered to 5 or 10 male Wistar rats /dose in the diet at dose levels of 0 (5 males) or 5000 (10 males) ppm (equivalent to 0, 444.3 mg/kg bw/day). No animals died during the study. Treatment-related piloerection and uncoordinated gait were observed in the treated animals. Treatment-related decreases in body weight and food consumption were observed in the treated animals. Blood analyses revealed a treatment-related increase in mean thromboplastin time, and treatment-related increases in mean alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase levels in the treated animals. Treatment-related increases in liver enzymes O-demethylase and N-demethylase were observed in the treated animals. Treatment-related increases in mean relative testes and brain weights and a treatment-related decrease in mean relative thymus weight were observed in treated animals. Necropsy revealed thymus diminished in size the treated animals. Microscopic examination revealed treatment-related cytoplasmic basophilia in the periportal hepatocytes, a treatment-related decrease in the medullary area of the thymus, reduction in the number of lymphocytes in both in the cortex and in the medulla of the thymus, and abnormal germ cells in the testes in animals treated with the test article. **The LOAEL and NOAEL cannot be determined from this study.** This 4-week oral toxicity study in the rat is a **range-finding study** and is a non-guideline study. It is unacceptable (see III.. C. below) and does not satisfy the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in the rat. (Corlett, 9/9/03)

52945-0076; 204819; P. Andrews and E. Sander. (2000). BSN 2060 Study for Subacute Oral Toxicity in Rats (Feeding Study for 4 Weeks). Bayer AG, Department of Toxicology, Wuppertal, Germany. Report No. 110804. (December 14, 2000). In a 4-week oral toxicity study (MRID 45854507), BSN 2060 (Batch No. NLL 5982-3, purity = 98.9%) was administered to 5 female Wistar rats /dose in the diet at dose levels of 0, 100, 500, or 5000 ppm (equivalent to 0, 10.9, 53.4, 536.3 mg/kg bw/day). No animals died during the study. Treatment-related piloerection, reduced motility, spastic gait, discolored feces, and increased reactivity when touched were observed at 5000 ppm. Treatment-related decreases in body weight and food consumption were observed at 5000 ppm. Blood analyses revealed treatment-related decreases in mean hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, platelet count, total protein, and cholesterol levels and treatment-related increases in mean thromboplastin time, mean alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase (not statistically significant), and urea levels at 5000 ppm. Treatment-related induction of some phase I liver enzymes (aldrin epoxidase and epoxide hydrolase) were observed at 500 and 5000 ppm. An increase in hepatic and renal cell proliferation based on PCNA analysis was observed at 500 and 5000 ppm (analysis not performed on animals in the 100 ppm group). Treatment-related increases in mean relative heart, kidney, and brain weights and treatment-related decreases in mean relative thymus, spleen, and ovary weights were observed at 5000 ppm. Necropsy revealed animals very thin in appearance at 5000 ppm. Microscopic examination revealed treatment-related increased follicular cell hypertrophy in the thyroid glands at 500 and 5000 ppm, and lymphoid atrophy in the spleen, thymus with an indistinct corticomedullary junction, and cytoplasmic change in the adrenal glands at 5000 ppm. **LOAEL (F) = 53.4 mg/kg/day (500 ppm). NOEL (F) = NOAEL (F) = 10.9 mg/kg/day (100 ppm)** based on the induction of phase I liver enzymes and increased follicular cell hypertrophy in the thyroid glands. This 4-week oral toxicity study in the rat is a **range-finding study** and is a non-guideline study. It

is unacceptable (see III. C. below) and does not satisfy the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in the rat. (Corlett, 9/22/03)

(90-day rat feeding study)

52945-0058; 204801; K.H. Leser and E. Sander. (2000). BSN 2060 Study on Subacute Toxicity in Wistar Rats (Dietary Administration over 3 Months with a Subsequent Recovery Period over 4 Months (sic)). Bayer AG, Department of Toxicology, Wuppertal, Germany. Report No. 109632. (January 31, 2000). In a 14-week oral toxicity study (MRID 45819515), BSN 2060 (Batch No. NLL 5982-11, purity = 99.5-100%) was administered to 10 Wistar rats/sex/dose in the diet at dose levels of 0, 100, 500, or 3000 ppm (equivalent to 0, 6.3, 31.7, 204.0 mg/kg bw/day in males and 0, 7.7, 36.6, 232.1 mg/kg/day in females) and 10 additional Wistar rats/sex (recovery group) in the diet at dose levels of 0 and 3000 ppm (equivalent to 0 and 209.4 mg/kg bw/day in males and 0 and 245.6 mg/kg bw/day in females). 3 females at 3000 ppm died (1 accidently) died during the study interval. Treatment-related clinical signs including staggering gait, bloody muzzle, hard feces, poor general condition, aggression, saltatory spasm (clonic), and squatting position were observed in females at 3000 ppm with clinical signs observed during the recovery period. A treatment-related decrease in body weight was observed in both sexes at 3000 (main group) with no decrease observed during the recovery period in the recovery group animals. A treatment-related increase in mean thromboplastin time in both sexes at 3000 ppm was observed; this effect was not observed in animals sacrificed following the recovery period. Treatment-related increases mean aspartate aminotransferase (females only), alanine aminotransferase (in both sexes), and alkaline phosphatase (in both sexes) levels at 3000 ppm and treatment-related decreases in mean cholesterol at 500 and 3000 ppm (in both sexes) and triglycerides levels at 3000 ppm (in both sexes) were observed; these effects were not observed in animals sacrificed following the recovery period except for the persisting increased mean alkaline phosphatase level in females. A treatment-related increase in the mean thyroid stimulating hormone level was observed in both sexes at 500 and 3000 ppm (not statistically significant in males at 500 ppm); this effect was not observed in animals sacrificed following the recovery period. Treatment-related increases in mean relative liver, kidney, and brain weights in both sexes at 3000 ppm, an increase in mean testes weight at 3000 ppm, and a treatment-related decrease in mean relative thymus weight in both sexes at 3000 ppm were observed with the decrease in thymus weight persisting in both sexes and the increase in kidney weight persisting in males following the recovery period. Microscopic examination revealed a treatment-related decrease in fat storage in the liver in both sexes at 3000 ppm with this decrease persisting in recovery group males, a treatment-related increase in follicular cell hypertrophy in the thyroid in males at 3000 ppm and in females at 500 and 3000 ppm with this increase persisting in recovery group females, treatment-related increased colloidal alteration in the thyroid in males at 500 and 3000 ppm and in females at 3000 ppm persisting in recovery group animals of both sexes, and atrophy of the thymus in both sexes at 3000 ppm persisting in recovery group animals of both sexes. **The LOAEL (M) is 31.7 mg/kg/day (500 ppm) and the LOAEL (F) is 36.6 mg/kg/day (500 ppm) based on liver and thyroid effects. The NOAEL (M) is 6.3 mg/kg/day (100 ppm) and NOEL (F) is 7.7 mg/kg/day (100 ppm).** This 90-day oral toxicity study in the rat is an acceptable, guideline study and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in the rat. (Corlett, 10/10/03)

(5-day repeated exposure rat inhalation study)

52945-0078; 204821; J. Pauluhn. (2000). Pilot Study on Subacute Inhalation Toxicity in Rats (Exposure: 5 x 6 Hours). Bayer AG, Department of Toxicology, D-42096 Wuppertal, Germany. Report No. 110811. (April 6, 2000). In a subacute inhalation toxicity study (MRID 45819605), Technical Grade BSN 2060 (batch nos. 06957/0009 (mixed batch), purity: 97.1%) was administered to 10 Wistar (Hsd Cpb:WU (SPF)) rats/sex/concentration by dynamic nose-only exposure at concentrations of 0, 11.2, 79.4, or 514.3 mg/m^3 (0, 0.0112, 0.794, 0.5143 mg/l) for 6 hours per day for 5 consecutive days. Five animals/sex/group were retained as a recovery cohort for an additional 11 days. The mean MMAD (GSD) values were 2.7 (1.9), 3.2 (1.8) and 3.2 (1.8) um for the 11.2, 79.4 and 514.3 mg/m^3 exposures, respectively. Exposure concentrations were determined by gravimetric analysis. Five females in the 514.3 mg/m^3 exposure group died between days 3 and 7. Clinical signs, which were monitored before and after exposure, were

observed only for the 514.3 mg/m^3 exposure group and included tremor, clonic-tonic convulsions, reduced activity, bradypnea, labored breathing, vocalization, avoidance reaction, giddiness, piloerection, limp, emaciation, cyanosis, squatted posture, apathy and salivation. These signs were more severe in the females and had disappeared by the afternoon of day 6. In the functional observational battery (FOB), performed on day 4, the 514.3 mg/m^3 females demonstrated reduced horizontal and vertical grip strength, reduced tonus, reduced corneal reflex and had no visual placing response. The males demonstrated no treatment-related effects in the FOB. Both sexes exhibited reduced mean body weights in the 514.3 mg/m^3 exposure group during the exposure group ($p<0.01$) with recovery evident for the males in this group by the end of the study. There were no apparent treatment-related effects on the hematology values. In the clinical chemistry, the mean serum ASAT, ALAT, alkaline phosphatase, and glutamate and lactate dehydrogenase activities were elevated for the 514.3 mg/m^3 females on day 7 (the increased activities were not statistically significant due to the wide variability in the individual values). The males were not affected. There was no treatment-related effect upon the liver enzyme activities or hepatic triglyceride content. The mean relative lung to body weight ratio was increased for both sexes in the high exposure group (males, $p<0.05$, females, $p<0.01$). For the spleen, the mean absolute, relative to body weight and relative to brain weight values were decreased for the 514.3 mg/m^3 group ($p<0.01$). In the gross examination, the thymus was reduced in size for both the males (2/5) and females (5/5) in the high exposure group. The spleen was reduced in size as well for the females in this group (4/5). The males in the high exposure group exhibited a greater incidence of dark red areas or foci in the lungs (0: 1/5 vs. high exp.: 4/5). Two females in the high exposure group also had bloated stomachs and pale livers. **The LOAEL is 0.5143 mg/L/day, based on the clinical signs and treatment-related lesions noted in this group. The NOAEL is 0.0794 mg/L/day.** This subacute inhalation toxicity study in the rat is a **range-finding study and does not satisfy the guideline requirement** for a subchronic inhalation study OPPTS 870.3465; OECD 413 in the rat. (Moore, 9/11/03)

(4-week repeated exposure rat inhalation study)

52945-0099; 204870; J. Pauluhn. (2001). Subacute Inhalation Toxicity on Rats (Exposure: 5 x 6 Hours/Week for 4 Weeks). Bayer AG, Department of Toxicology, D-42096 Wuppertal, Germany. Report No. G200121. (October 2, 2001). In a subacute inhalation toxicity study (MRID 45819622), Technical Grade BSN 2060 (purity: 97.4%, batch no. 06957/0009) was administered to 10 Wistar rats/sex/concentration by dynamic nose-only exposure at concentrations of 0, 5.0, 24.6, or 80.9 mg/m^3 for 6 hours per day, 5 days/week for up to a total of 22 days over a 30 day period (0, 0.005, 0.0246, and 0.0809 mg/l). The mean MMAD (GSD) values were 2.70 (1.91), 2.71 (1.77) and 2.87 (1.81) um for the 5.0, 24.6 and 80.9 mg/m^3 exposure groups, respectively. An additional 10 animals/sex/group were included in the control and high exposure groups and maintained for a recovery period of approximately 5 weeks. No deaths occurred during the study. No treatment-related clinical signs were noted over the course of the exposure or recovery periods. These data included an evaluation of breathing patterns, neurological reflexes, foot splay and colonic temperature. In the hematology evaluation, the blood clotting time was increased for the 24.9 and 80.9 mg/m^3 females after 4 weeks of exposure ($p<0.01$ or 0.05). This effect was not evident after 5 weeks of recovery. In the clinical chemistry, the serum alkaline phosphatase activity was increased for the 80.9 mg/m^3 females after 4 weeks of exposure ($p<0.05$). Likewise, this effect was not evident after the recovery period. Among the liver enzymes which were evaluated, N-demethylase and O-demethylase activities were increased in the 24.9 and 80.9 mg/m^3 after 4 weeks of exposure $p<0.01$ or 0.05) with recovery 5 weeks later. There were no treatment-related lesions or effects on organ weights noted in the necropsy or histopathological evaluations. **The NOAEL is 0.0809 mg/L/day, based on the lack of any toxicologically significant effects in the highest exposure group.** This subacute inhalation toxicity study in the rat is a **non-guideline, range-finding study** for the longer term subchronic inhalation toxicity study and does not satisfy the guideline requirement for a subchronic inhalation study OPPTS 870.3465; OECD 413 in the rat. (Moore, 9/17/03)

(4-week rat repeated dose dermal toxicity study)

52945-0075; 204818; F. Krotlinger and A. Popp. (2001). BSN 2060 Study for Subacute Dermal Toxicity in Rats (Four-Week Treatment Period). Bayer AG, Department of Toxicology, Wuppertal, Germany. Report No. 110802. (March 22, 2001). In a 28-day dermal toxicity study (MRID 45819603), BSN 2060 (Batch No. 06957/0009, purity = 97.3%) was applied to the shaved skin of 10 Wistar rats/sex/dose at dose levels of 0, 100, 300, and 1000 mg/kg bw/day, 6 hours/day for 5 days/week (7 days/week during the last week) during a 28/29-day period. There were no compound related effects in mortality, clinical signs, body weight, food consumption, hematology, organ weights, or gross and histologic pathology. There was a treatment-related decrease in the mean cholesterol level in females at 1000 mg/kg/day. **The LOAEL (F, systemic) is 1000 mg/kg/day, based on a decrease in mean cholesterol level. The NOAEL (M, systemic) is 1000 mg/kg/day based on no effects at the highest dose tested, the NOAEL (F, systemic) is 300 mg/kg/day based on a decrease in the mean cholesterol level, and the NOAEL (M/F, skin) is 1000 mg/kg/day based on no effects at the highest dose tested.** This 28-day dermal toxicity study in the rat was originally determined to be an unacceptable guideline study (Corlett, 11/24/03). Subsequently it was upgraded to **acceptable** with submission of data (e-mail from Dr. Michael Dreist, dated 4/15/04) ensuring sufficient moistening of the test article before application to the test animals.

(immunotoxicity study)

52945-0084; 204855; F. Krotlinger and H.-W. Vohr. (2001). BSN 2060: Plague-Forming-Cell Assay in Rats (Feeding Study over about 4 Weeks. Bayer AG, Department of Toxicology, D-42096 Wuppertal, Germany. Report No. 110821, July 2, 2001. In an immunotoxicity study (MRID 45819608), Technical Grade BSN 2060 (purity: 97.4%, batch no. 06957/0009) was administered to 8 HsdCpb:WU rats/sex/dose in the diet at dose levels of 0, 100, 500 or 3000 ppm (equivalent to 0, 9.6, 52.8, 291.6 mg/kg bw/day for males and 0, 10.7, 45.7, 288.6 for females) for 4 weeks. Two of the females in the 3000 ppm treatment group died during the treatment period. No clinical signs were evident for the both sexes in 100 ppm group and the males in the 500 ppm treatment group. Discolored feces were evident for the 500 and 3000 ppm females and the 3000 ppm males. For both sexes in the 3000 ppm group, piloerection was noted. In addition, the 3000 ppm females demonstrated increased motility, aggression and clonic saltatory spasm (two animals). The mean body weights of both sexes in the 3000 ppm treatment group were lower than those of the control over the course of the study ($p<0.01$). Food consumption on a per weight basis was actually increased for this treatment group ($p<0.05$). In the Plaque-Forming Cell Assay, in which the animals were treated with sheep erythrocytes by iv injection 5 days prior to being euthanized, the number of cells/spleen was significantly lower for the 3000 ppm males and the 500 and 3000 ppm females. The number of antibody-plaque forming cells (AFC)/10^6 spleen cells was not affected in a treatment-related manner. The number of AFCs per spleen reflected the reduced number of cells/spleen. The spleens were not weighed and no histopathology was performed on the tissue to document whether the spleens were affected by the treatment in a non-immunotoxic manner. The data suggest that under the conditions of this study, the test material did not suppress the humoral immune response in a dose-dependent manner. Treatment did not significantly alter the IgM antibody-forming cell response to the T-dependent antigen, sheep erythrocytes. The assay was deficient in that no positive control was performed in conjunction with the assay. The NOAEL for clinical signs was established at 500 ppm, based on the signs demonstrated by the animals in the 3000 ppm treatment group. A NOAEL for immunotoxicity could not be established due to the inadequacies of the study. This immunotoxicity study is classified as an **unacceptable guideline study** that does not satisfy the guideline requirement for an immunotoxicity study (OPPTS 870.7800) in the rat . The Plaque Forming Cell Assay constitutes only one area of testing which must be included in the study. 1) The other recommended assays are natural killer cell activity and enumeration of total B cells, total T cells, and T cell subpopulations in the spleen or peripheral blood. These assays were not included in the study. 2) In addition, weights of the thymus and spleen were not recorded. The data for the Plaque-Forming Cell Assay do not indicate an immunotoxic response. (Moore, 10/1/03)

(4-week mouse feeding study)

52945-0066; 204809; L. Schladt. (1999). BSN Range-Finding Subacute Toxicity Study in CD-1 Mice (Administration in the Feed over 28 Days). Bayer AG, Department of Toxicology,

Wuppertal, Germany. Report No. 109640. (August 2, 1999). In a 4-week oral toxicity study (MRID 45819523), BSN 2060 (Batch No. NLL 5982-11, purity = 99.5-100%) was administered to 3 Crl:CD-1(ICR)BR mice/sex/dose in the diet at dose levels of 0, 1000, 3500, or 7000 ppm (equivalent to 0, 202.3, 720.0, 1292.3 mg/kg bw/day in males and 0, 269.6, 699.2, 1706.0 mg/kg bw/day in females). At 7000 ppm, 2 males and 2 females died during week 3, and 1 male and 1 female died during week 4. No clinical signs were reported. A treatment-related decrease in mean body weight gain was observed in both sexes at 1000 and 3500 ppm and a treatment-related mean weight loss was observed in both sexes at 7000 ppm. One female that died exhibited dark red lungs and a filled stomach. No abnormalities were reported in the surviving animals. **The LOAEL (M) is 202.3 mg/kg/day (1000 ppm) and the LOAEL (F) is 269.6 mg/kg/day (1000 ppm) based on decreased mean body weight gain. The NOAEL was not determined.** This 4-week oral toxicity study in the mouse is a range-finding study and is a non-guideline study. It is unacceptable (see III. C. below) and does not satisfy the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in the mouse. (Corlett, 10/10/03)

(90-day mouse feeding study)

52945-0069; 204812; L. Schladt and E. Sander. (1999). BSN 2060 Study on Subchronic Toxicity in CD-1 Mice (Dietary Administration Over 3 Months). Bayer AG, Department of Toxicology, Wuppertal, Germany. Report No. 109643. (September 14, 1999). In a 3-month oral toxicity study (MRID 45819526), BSN 2060 (Batch No. NLL 5982-11, purity = 99.5%) was administered to 10 Crl:CD-1(ICR)BR mice/sex/dose in the diet at dose levels of 0, 140, 700, or 3500 ppm (equivalent to 0, 21.7, 104.5, 589.3 mg/kg bw/day for males and to 0, 35.3, 190.5, 1010.3 mg/kg bw/day for females). No animals died during the study. No treatment-related clinical signs of toxicity were reported. Treatment-related decreases in body weight and food consumption were observed in females at 3500 ppm. Blood analyses revealed treatment-related decreases in the mean hemoglobin level in both sexes at 700 and 3500 ppm, the mean corpuscular hemoglobin level in both sexes at 3500 ppm, and the mean cholesterol level in females at all dose levels, and treatment-related increases in the mean alanine aminotransferase level at 700 and 3500 ppm and the mean alkaline phosphatase level at 3500 ppm in males. A treatment-related increase in mean relative liver weight was observed in males at 3500 ppm. Necropsy revealed discolored adrenal glands in both sexes at a dose-related frequency. Microscopic examination revealed a treatment-related decrease in fine vesiculation of the adrenal glands and the treatment-related presence of cytoplasmic eosinophilia in zona fasciculata cells of the adrenal glands in both sexes at all dose levels. **LOAEL (M) = 21.7 mg/kg/day (140 ppm), LOAEL (F) = 35.7 mg/kg/day (140 ppm), NOAEL cannot be determined** based on discolored adrenal glands, a decrease in fine vesiculation of the adrenal glands, and the presence of cytoplasmic eosinophilia in zona fasciculata cells of the adrenal glands in both sexes. This study is unacceptable (see III. C. below) and does not satisfy the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in the mouse. (Corlett, 11/5/03)

52945-0073; 204816; Schladt and E. Sander. (2001). Study on Subchronic Toxicity in CD-1 Mice (Dietary Administration Over 14 Weeks). Bayer AG, Department of Toxicology, Wuppertal, Germany. Report No. 110800. (February 22, 2001). In a 14-week oral toxicity study (MRID 45819601), BSN 2060 (Batch No. 06957/0009, purity = 97.0-97.6%) was administered to 10 Crl:CD-1(ICR)BR mice/sex/dose in the diet at dose levels of 0, 20, or 80 ppm (equivalent to 0, 3.2, 11.5 mg/kg bw/day for males and to 0, 5.1, 20.3 mg/kg bw/day for females). No animals died during the study. No treatment-related clinical findings were observed. No treatment-related effects on body weight, food consumption, or hematology were observed. Blood analyses revealed a treatment-related decrease in the mean cholesterol level in both sexes at 80 ppm. No treatment-related effects on organs weights were observed. Necropsy revealed no treatment-related abnormalities. Microscopic examination revealed cytoplasmic eosinophilia in the adrenal glands in 1/10 female animals at 80 ppm. **LOAEL (M) = 11.5 mg/kg/day (80 ppm) and LOAEL (F) = 20.3 mg/kg/day (80 ppm)** based on decreased mean serum cholesterol levels (in both sexes) and the presence of cytoplasmic eosinophilia in the adrenal glands (in females only). **NOAEL of 3.2 mg/kg/day (20 ppm) in males and a NOAEL of 5.1 mg/kg/day (20 ppm) in females**. This study is unacceptable (see III. C. below) and does not satisfy the guideline

requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in the mouse. (Corlett, 11/13/03)

(90-day dog feeding study)

52945-0091; 204862; Detzer, K. and E. Sander, "BSN 2060: Subchronic oral toxicity study in dogs," Bayer AG, Department of Toxicology, Wuppertal, Germany. Laboratory Project ID: Bayer Corporation Agricultural Division Report No. 110922, Bayer AG Study No. T2067577. (May 22, 2001). In a 90-day oral toxicity study (MRID 45819614). Spiromesifen (97.4% purity, Mixed batch No. 06957/0009) was administered to four beagle dogs/sex/dose in diet at dose levels of 0, 20, 50, 250, or 2000 ppm (equivalent to 0, 0.75, 1.85, 9.2, or 71 mg/kg bw/day). Treatment throughout the dosage range tested was well tolerated: there were no effects on clinical signs, body weight, food consumption, reflexes, EKG's, or ophthalmology. Marked, dose-related increases in liver metabolic enzyme activities were noted at 250 and 2000 ppm for activities of aminopyrine-N-demethylase (about 2-fold and 4-fold, respectively) and p-nitroanisole-O-demethylase (about 1.5-fold and 2-fold, respectively), and for 7-ethoxycoumarin deethylation activity (about 3-fold and 4- to 6-fold, respectively). Epoxide hydrolase activity in homogenized liver was marginally increased at 250 ppm (34% increase in males, 86% in females) and substantially increased (over 2-fold) at 2000 ppm. Cytochrome P-450 levels were elevated in 250 ppm females (33%) and at 2000 ppm in both sexes (2-fold). These findings are consistent with enhanced liver metabolic capacity, and with liver cytoplasmic change (centrilobular hepatocytes had cytoplasm considered "more homogeneous and dense" than control tissue). This was the only histopathology clearly attributable to treatment in this study. Incidences were 0, 0, 0, 3, and 3 in controls through increasing dose groups in males, and 0, 0, 0, 2, and 2 in corresponding females (N = 4 in all cases). Thyroxine (T4) levels in 250 ppm females were reduced by an average of 33% during the treatment phase. There was no gender difference in T4 levels in 2000 ppm dogs, which averaged a 63% reduction during treatment compared to controls. These changes were plausibly due to increased liver metabolism. All other effects were observed only in 2000 ppm dogs. These included absolute liver weight increases of 15% and 21% in males and females, respectively, as well as elevated plasma alkaline phosphatase activity (average 3-fold increase) and a minor increase in γ-glutamyl transferase (GGT) activity, both of which enzyme changes are associated with cholestasis, which could result from hepatocellular hypertrophy. Additional findings at 2000 ppm were elevated liver levels of triglycerides (about 80% increase), plus about 2-fold increased levels of metabolic enzyme activities in liver tissue for aldrin epoxide and UDP-glucuronyl transferase. In a brief elective metabolic disposition assay, assessment of plasma samples taken 22 hr after dosing during week 12 revealed no detectable parent compound, but BSN 546 (the enol product of ester bond cleavage) was quantifiable at 250 and 2000 ppm. In that range, detected levels rose only about 3-fold for an 8-fold difference in administered dose. Mean plasma concentrations in 2000 ppm males and females were 31 and 41 nmol/ml, respectively. **The LOAEL of 250 ppm and associated NOAEL of 50 ppm (respectively equivalent to 9.2 mg/kg/day and 1.85 mg/kg/day: no notable sex differences) were based upon the above effects, which indicated liver as the primary target organ.** The 4-week pilot study by the same investigators (MRID 45854506) evaluated effectively the same set of parameters, and is briefly reviewed as an appendix to this 90-day study. The 4-week study found liver metabolic activity toward several substrates at 100 ppm (3.8 mg/kg/day) but not at the next lower dose of 25 ppm (0.92 mg/kg/day), suggesting that the NOAEL for the subchronic study was close to a level eliciting a measurable response. This 90-day oral toxicity study in the dog is acceptable (guideline) and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3150; OECD 409) in non-rodent species. This study also provided a basis for dose selection for the subsequent chronic dog study. (Aldous, 11/12/03)

52945-0100; 204871; J. Ruf and Chr. Ruhl-Fehlert. BSN 2060: Subchronic Toxicity Study in Beagle Dogs (13 Week Feeding Study). Bayer AG, Department of Toxicology, D-42096 Wuppertal, Germany. Report No. G200127. November 8, 2001. In a 90-day oral toxicity study (MRID 45819623), BSN 2060 Technical (purity: 97.6%, batch no. 06957/0009) was administered to 4 beagle dogs/sex/dose in the diet at dose levels of 0, 3000 or 5000 ppm (equivalent to 0, 98.4, 172.5 mg/kg bw/day for males and 0, 102.8, 170.8 mg/kg bw/day for females) for 13 weeks. There were no treatment-related effects on the mean body weights, body weight gain, food

consumption or the various physiological parameters which were evaluated (reflexes, body temperature, blood pressure, heart rate, and electrocardiogram). Ophthalmoscopy, hematology and urinalysis did not indicate any treatment-related effects. In the clinico-chemical evaluation, the alanine aminotranferase and alkaline phosphatase activities were elevated for both sexes in both treatment groups. The liver enzymes, N- and O-demethylase, Cytochrome P450, 7-ethoxycoumarin deethylase, aldrin epoxidase, and epoxide hydrolase had elevated activity levels for both of the treatment groups. There was an increase in both the mean absolute and mean relative liver weights for both treatment groups. In the histopathological examination, predominantly moderate hepatocellular hypertrophy, cytoplasmic change and a minimal to slight increase in lipid content was noted for animals in both of the treatment groups. Minimal to slight hepatocellular degeneration was observed in one animal of both treatment groups. A minimal to slightly increased cortical vacuolation in the adrenal glands was observed in one animal in both treatment groups. Although serum thyroxine levels were decreased for the treated animals throughout the study, there were no other effects which directly indicated thyroid toxicity. The mean absolute and relative thyroid weights were not affected in a treatment-related manner. There was no treatment-related histopathology in the thyroid and the other thyroid-related clinical parameters (triiodothyronine, thyroxine-binding index, thyroxine-stimulating hormone) were not apparently affected by the treatment. Histopathological examination of the epididymides of the males in the two treated groups revealed an increased incidence of cellular debris (4 of 8 animals) and oligospermia in one animal of the 5000 ppm group. The mean absolute and relative epididymal weights were lower in a dose-related manner. The study authors attributed these results to the sexual immaturity of the test animals even though the controls animals did not demonstrate similar effects. The increasing concentration of the metabolite in the plasma indicated an extended half life for the parent compound. The target organ was the liver. These study data were not adequate to establish a LOAEL or a NOAEL. The apparent purpose of the study was to demonstrate marked treatment-related effects for the test material. This 90-day oral toxicity study in the dog is **supplemental and does not satisfy the guideline requirement for a 90-day oral toxicity study** (OPPTS 870.3150; OECD 409) in a non-rodent species. The results of this study should be considered in conjunction with the results of another 90-day dog oral toxicity study (MRID 45819614). (Moore, 12/2/03)

METABOLISM STUDIES

52945-0007; 204716; D. Shaw. (2000) BSN 2060: Metabolism in Rats. Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, PE28 4HS, England. Report No. 110331. October 24, 2000.

In a metabolism study (MRID 45819403), BSN 2060 ([Dihydrofuranone-3-^{14}C] BSN 2060, radiochemical purity: >97%, specific activity: 146.8 uCi/mg, lot no. 12513/1, non-labeled BSN 2060, purity: 99.5%, lot no. M00391) was administered to Wistar, Crl(W1)BR VAF/Plus rats orally by gavage. The study consisted of nine individual treatment protocols. In the excretion-balance portion, a preliminary study was performed in which 4 animals/sex were treated with 500 mg/kg of the test material. In 3 additional treatments, 4 animals/sex (unless otherwise noted) were treated with a single dose of 2 mg/kg, a single dose of 500 mg/kg (males only) or 14 daily unlabeled doses of 2 mg/kg each followed by a single dose of radiolabeled 2 mg/kg on the 15th day. Urine and feces were collected at specified intervals up to 72 hours post-dose. In the preliminary test, expired air was collected as well for the same time interval. In the 3 blood/plasma pharmacokinetics protocols, 12 animals/sex (unless otherwise noted) were dosed with a single dose of 2 mg/kg, a single dose of 500 mg/kg (males only) or 14 daily unlabeled doses of 2 mg/kg each followed by a single dose of radiolabeled 2 mg/kg on the 15th day. Blood was collected from 4 animals/sex at specified time points up to120 hours post-dose. In the biliary excretion study, 4 males whose bile ducts had been cannulated received a single dose of 2 mg/kg and bile, urine and feces were collected at specified time points up to 48 hours post-dose. In the whole body autoradiography study, 6 males received a single dose of 2 mg/kg and one animal was euthanized at each specified time point up to 72 hours post-dose. For a single dose of 2 mg/kg, 39% of the administered dose was excreted in the urine and 55 to 57% in the feces with 88 to 90% of the dose being eliminated within the first 24 hours. Treatment with multiple doses of 2 mg/kg did not affect the ratio of radiolabel excreted in the urine and feces. Concentrations of

residual radioactivity in the tissues were quite low at 72 hours post-dose. Treatment with 500 mg/kg of the test material resulted in a much lower percentage of the administered dose being excreted in the urine (7 to 9%) with the remainder recovered in the feces. Following a single dose of 2 mg/kg, the test material was rapidly if incompletely absorbed with the Cmax value in the blood achieved within 1 to 2 hours post-dose. Treatment with multiple doses of 2 mg/kg or a single dose of 500 mg/kg delayed the Tmax to 3 to 4 hours and 6 hours, respectively. The Cmax values and the concentration versus time curves [AUC(t)] indicated a disproportionately lower increase in the uptake of the radiolabel into the blood between the 2 mg/kg and 500 mg/kg treatments. At 500 mg/mg, these values were approximately 80% less than would be predicted by a proportionately linear increase. These data confirmed the reduced percentage of radiolabel which was absorbed at the 500 mg/kg treatment level in the excretion-balance profile. In the two 2 mg/kg treatment regimens, the Cmax and AUC(t) values for the females were less than those of the males with values ranging from 66 to 86% for Cmax and 43 to 55% for AUC(t) in comparison to the males. These data indicated that the females experienced less of an exposure to the test material than did the males. The whole-body autoradiograms qualitatively demonstrated the distribution of the radioactivity throughout the body. The highest areas of concentration at 1 hour post-dose were the gastrointestinal tract, bladder and blood within the heart. Overall tissue distribution appeared to be the highest at 4 hours post-dose with a progressive diminishment over the time-course of the study. At 48 hours post-dose, observable levels of radioactivity were present only in the gastrointestinal tract, kidneys and bladder. The test material was initially metabolized to the keto-enol by loss of the dimethylbutyric acid moiety. Both the phenyl and cyclopentyl rings were hydroxylated and the methyl groups on the phenyl ring were ultimately oxidized to a carboxylic acid. These metabolites were largely recovered in the bile and urine. The predominate moiety recovered in the feces was the unmetabolized test material. No conjugation with either glucuronic acid or sulphate was observed. **This metabolism study in the rat is classified acceptable (guideline) and satisfies the guideline requirement for a metabolism study [OPPTS 870.7485, OECD 417] in the rat.** (Moore, 10/27/03)

52945-0170; 204964; C. A. Sebesta. 2002. An Exploratory Study to Determine the Rate and Route of Elimination of BSN 2060-Phenyl-UL-^{14}C When Administered Intravenously or Dermally to Male Rheus Monkeys. Charles River Laboratories, Discovery and Development Services, Worcester, MA. Report No. G200207. June 27, 2002. In a metabolism study (MRID 45819828), BSN 2060-Phenyl-UL-^{14}C (batch no. not provided, radiochemical purity: 100%, specific activity: 34.9 mCi/mmol) was administered intravenously to one male rhesus monkey at a dose level of 0.231 mg/kg (21.8 uCi/kg). In a second part of the study, BSN 2060 SC 480 (batch no. K1 BRD (0037), a.i. 45.2%) containing BSN 2060-Phenyl-UL-^{14}C, was applied to the skin of one male rhesus monkey at a dose of 0.191 mg/kg or 18.3 ug/cm^2 (18.0 uCi/kg) for 8 hours. Dosing via the intravenous route resulted in the excretion of the radiolabel largely in the urine. The percentage of the administered dose which was recovered in the urine, feces and cage debris/rinse samples was as follows: urine (54.32%), feces (13.08%) and cage debris/rinse (26.57%). A significant fraction of the dose was recovered in the cage debris/rinse. Although it was not definitive whether this radiolabel was from the urine or feces, a greater part of that fraction was recovered prior to any radiolabel being found in the feces. The 13% of the dose which definitively was recovered in the feces had likely passed through the biliary excretion route. Excretion was relatively rapid with greater than 70% of the administered dose recovered within the first 24 hours. Dermal application of the test material resulted in only limited absorption for the 8 hour exposure period, 3.31%. A large fraction of that total was recovered from the urine and cage debris/rinse. This metabolism study in the monkey is classified as **supplemental (non-guideline)** and does not satisfy the guideline requirement for a metabolism study [OPPTS 870.7485, OECD 417]. The study is exploratory in nature and does not comply with the guideline requirements. (Moore, 10/31/03)